NOAA-NATIONAL MARINE FISHERIES SERVICE

SOUTHEAST FISHERIES SCIENCE LABORATORY IN CHARLESTON

FY97 SIGNIFICANT ACCOMPLISHMENTS

MARINE BIOTOXINS PROGRAM

Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trials. The first of these trials, testing the assay for NSP in oysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY98.

Reporter gene assays have been established using the c-fos response element linked to the coding region for firefly luciferase, and this approach has been used to design detection methods for ciguatoxins and *Pfiesteria* toxins. This method is effective for measuring ciguatoxins in barracuda and should permit high capacity monitoring of the toxin in small finfish samples. A second reporter gene assay has been developed for *Pfiesteria* toxin in collaboration with the NIEHS and North Carolina State University.

Highly sensitive and efficient HPLC-MS protocols have been developed for rapid identification and quantification of brevetoxin (PbTx), ciguatoxin (CTX), and okadaic acid (OA), as well as their analogs. This methodology has been optimized to characterize and quantitate these toxins accurately in subnanomolar concentrations. Chromatographic methods as front ends to mass spectrometry have been developed to concurrently allow matrix independent analyses of these toxins. These are being implemented to circumvent tedious multiple extractions/pre-purification steps making for more rapid and efficient testing protocols.

Studies on growth regulation in dinoflagellates have focused this year on the role of endogenous cellular rhythms in the initiation, growth, maintenance, and decline of harmful algal blooms. Field studies using flow cytometry have confirmed that cell division in a naturally occurring bloom of the Florida red tide organism, *G. breve,* is phased to the diel cycle. The cell division rate in the bloom population was 0.2 div./day, similar to division rates obtained in the laboratory. No correlation was found between vertical distribution and cell division. Identification of the biochemical mechanisms controlling cell growth will advance prediction of the dynamics of harmful algal blooms.

Pseudo-nitzschia spp. were found to be abundant in Louisiana coastal samples. At shelf sites these species were present in 70% of all samples and concentrations were maximal in spring, often exceeding one million cells/liter. Among those species identified were the toxic *P. multiseries, P. delicatissima, and P. pseudodelicatissima*. Future work is aimed at addressing the issues of how environmental factors influence DA production by *Pseudo-nitzschia* spp. in natural populations, and why no outbreaks of DA poisoning have occurred in this region.

The PSP receptor binding assay (corroborated by HPLC analyses) is being used to study toxin transfer in Gulf of Maine food webs. Work to date has shown that PSP toxins move preferentially from their algal producers (*Alexandrium* spp.) into the larger size fractions of the zooplankton grazing community dominated by large copepods, even though these animals were not numerically dominant. Toxin-accumulating copepods could provide a direct trophic linkage for vectorial intoxication and possible mortality of planktiviorous fish as well as endangered whales which are known to feed upon these copepods.

An egg microinjection method has been developed to provide a quantitative measure of the cumulative effects of toxins on population declines in the marine environment that result from subtle reductions in larval viability. This method has been used to determine the levels of ciguatoxin transferred to the egg during spawning that reduces viability. This information will allow fishery managers to better predict the adverse effects of CTXs on populations of commercially valuable species of reef fish.